47. The use according to claim 43, wherein an export gene from Corynebacterium is utilized.

48. The use according to claim 43, wherein Corynebacterium is used as amino acid producing microorganism.

REMARKS

Table 1 on page 16 has been deleted because the information presented therein is identical to that of SEQ ID No: 3 from nt 1421-2293.

Table 2 on pages 17 -19 has been deleted because the information presented therein is identical to that disclosed in SEQ ID No: 1.

Table 3 on page 20 has been deleted because the information presented therein is identical to SEQ ID No: 2.

Rejection under USC 112, first paragraph:

In claim 1, the phrase "including any allele variation thereof" has been deleted in order to overcome the Examiner's doubts. In this connection, it is noted with regard to the Examiner's comment on page 4, last paragraph of the Official Action, that the present invention pertains to a microbial process. Microorganisms in the sense of the present invention are prokaryotes and prokaryotes are not diploid, but haploid organisms.

Rejection under USC 112, second paragraph:

The references to the sequence identifications including the letters (A, B, C) have been changed to expression in which the letters (A, B, C) have been deleted in accordance with the PatentIn sequence listing. A new sequence listing is enclosed herewith together with a paper copy thereof for a better understanding. The information recorded in computer readable form is identical to the written sequence listing. The sequence listing does not go beyond the disclosure in the original application.

For correctly identifying the sequences cited in the claims and the corresponding description, in the claims and the description, the following changes have been made:

a) SEQ ID No. (A) 2 has been changed to SEQ ID No. 2, in accordance with the PatentIn sequence listing on file corresponding to the amino acid sequence of the export carrier protein LysE.

b) The reference to table 3 has been deleted because it is identical to SEQ ID No. 2, that is, it includes no additional information.

c) SEQ ID No. (A)1 has been changed to SEQ ID No. 1 in accordance with the sequence listing on file corresponding to the nucleotide sequence from nucleotide 1016 – 1726 of the export carrier genet Lys E.

d) The reference to Table 2 in claim 1 has been deleted because it is identical to the information disclosed in SEQ ID No. 1.

e) SEQ ID No. (B)3 has been changed to SEQ ID No 3 in accordance with the PatentIn sequence listing LysG orf 3. app (on file) corresponding to the complement nucleotide sequence (antisense strand) which encodes from nucleotide 2 – 652 an orf3 and from nucleotide 1421 – 2293, the regulator gene Lys G. The amino acid sequence encoded by nucleotide 1421 – 2293 of SEQ ID No. 3 is identical to the information in table 1.

It is believed that claim 1 as amended overcomes the Examiner's objection when taking the above comments into consideration.

Claims 2, 3, and 4 appear to be quite definite.

The Examiner has stated that "claims 2, 3, 4 are indefinite because [they] refer to increased endogenous export activity" and she asks "compared to what is the activity increased?". The activity of the export carrier is increased of course in comparison with the endogenous, that is, the naturally occurring export carrier activity in a microorganism. This is common language use in the field. The endogenous export carrier activity can be measured with routine methods by a person skilled in the art, and in each microorganism, is selected so as to be useful for the production of amino acids according to the invention. Therefore, the endogenous activity is quite definite. And "increased export activity" is clearly an activity

higher than the natural endogenous activity. The activity is increased by either of the step i) – iv) of claim 1.

Procedures how to increase the natural (endogenous) export carrier activity are consequently also described in a clear and definite way.

With regard to claims 4 and 5, it is not quite clear to Applicants what the Examiner's objections are: Claim 4 pertains to the export gene expression, which is increased by increasing the numbers of gene copies. The wording that "the export carrier is increased by increasing the number of gene copies" is clear and it is evident for a person skilled in the art that the gene of interest is not only installed in increased numbers, but also that the additional gene copies are expressed. Nevertheless, claim 4 has been amended by adding the phrase "whereby the export carrier gene is expressed from the additional copies " (claim 4).

Claim 5 defines that the export gene is installed in a gene construct to provide for the increase in the number of copies.

However, an increased export carrier can also be achieved in other ways. As defined in claim 6, the export carrier gene is installed on a vector, which is replicated with a low number of copies (e.g. 1 – 10 copies). This makes sense since an increased number of gene copies is to be understood as being more than one copy. More than one additional copy of a single naturally occurring gene (e.g. two copies or even three or ten copies) of the export carrier gene result in an increased activity in relation to the basic activity (encoded through only one genomic gene copy).

This preferred variation of the invention was claimed taking into consideration that the export carrier gene encodes a protein, which is a membrane protein. Some cases have become known in the art concerning the over-expression of membrane proteins wherein the cell membrane was negatively affected by an increased number of export membrane proteins and, at worst, the cells died.

This, however, is only a possibility; it is not a compelling scenario. Consequently, an increased export carrier activity may also be reached by an increase of the number of copies of the export gene beyond 10, even to several hundred copies (which of course will be ex-

pressed). Thus, low numbers of copies as well as high numbers of copies of the export gene are useful to increase the export carrier activity.

It is believed that the above comments answer the Examiner's doubts concerning claim 6.

In claim 7, in view of the Examiner's objections, "assigned" has been changed to "operably linked". "Operably linked" is intended to mean that the nucleotide sequence of interest is linked to regulatory sequence(s) in a manner which allows for the expression of the nucleotide sequences to be used with each other so that both sequences fulfill their proposed function. For example, a promoter or an enhancer, etc... A person skilled in the art knows that the regulatory sequence, for example, the promoter used, should be operatively linked to the nucleic acid such that the promoter causes transcription of the nucleic acid which results in the synthesis of an RNA, which encodes a polypeptide. A corresponding disclosure, in an implicit manner, is provided on page 5, lines 9 – 22 and page 6, lines 9 – 12 of the description. It is assumed that this overcomes the Examiner's concerns with respect to claim 7.

Claim 8 has been rejected by the Examiner because she feels it is unclear how or where there is a definition of a regulatory sequence which includes a segment that is a coding domain.

In response, it is noted that one example for a regulatory sequence may be the instant regulatory protein LysG. This protein LysG may be involved in the expression control of the export carrier gene LysE. Further, the LysG regulator protein is encoded by the nucleotide sequence SEQ ID No. 3 from nt 1421 – 2293 (=table 1) in the enclosed PatentIn sequence listing.

In a preferred embodiment of the invention, the regulatory sequence can be operably linked to the export carrier gene, whereby the regulatory sequence itself is a coding sequence which is transcribed into mRNA and this mRNA is translated into a protein, namely the regulator protein LysG. Then the LysG regulates the expression (probably by interaction with the RNA polymerase transcription machinery) of the coding sequence encoding the carrier LysE. Another example of a regulatory sequence known in the art, which comprises a coding domain is the lacI repressor gene of the lacZ operon in the bacterium Escherichia coli. From

the above comments, it should be apparent that the subject matter as defined in claim 8 is clear to a person skilled in the art.

Claims 1 – 20 have been made properly dependent.

Claim 43 has been amended to include the process steps necessary for the microbial production of amino acids.

Claims 44 and 45 have been cancelled.

Claims 46 to 48 depend on claim 43 in which all necessary method steps are included.

Consequently, also these claims include the respective steps so that they should no longer be objectionable.

Reconsideration and allowance of claims 1 to 8, 10 – 20, 43 and 46 to 48 is solicited.

Respectfully submitted,

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MARKUP VERSION TO SHOW CHANGES MADE

In the Specification:

Page 8, first full paragraph:

A multitude of sequences is known which code for membrane proteins of unknown function. By providing in accordance with the invention export genes such as the export gene with the nucleotide sequence of nucleotide 1016 to 1726 in accordance with SEQ ID No. [(A)1] 1 and table 2 or respectively, the corresponding export proteins for example that with the amino acid sequence according to SEQ ID No.[(A)2] 2 [and table 1], it is now possible to identify by sequence comparison membrane proteins, whose function is the transport of amino acids. The export gene identified in this way can subsequently be used to improve the amino acid production in accordance with the process of the invention.

Page 8, second full paragraph:

The membrane proteins known from the state-of-the-art generally include 12, some also only 4 transmembrane helices. However, it has now been found surprisingly that the membrane proteins responsible or suitable for the export of amino acids include 6 transmembrane helices (see for example, the amino acid sequence of an export protein listed in SEQ ID No.[(A)2] 2 and table 3, wherein the 6 transmembrane areas have been highlighted by underlining). Consequently, there is a new class of membrane proteins present which has not yet been described.

Page 11/12 bridging paragraph:

The nucleotide sequence of the 2.3kb BamH1 fragment was performed according to the dideoxy-chain termination method of

Sanger et al. (Proc. Natl. Acad. Sci USA(1977) 74:5463-5467) and the sequencing reaction with the Auto Read Sequencing kit from Pharamcia (Uppsala, Sweden). The electrophoretic analysis occurs with the automatic laser-fluorescence DNA sequencing apparatus (A.L.F) from Pharmacia-LKB(Piscataway, NJ, USA). The nucleotide sequence obtained was analyzed by the program packet HUSAR (Release 3.0) of the German Cancer Research Center (Heidelberg). The nucleotide sequence and the result of the analysis is presented in SEQ ID No [(A)]1 and Fig. 2. The analysis results in two fully open reading frames (ORF) on the sequenced DNA piece. ORF1 codes for a protein with a length of 236 amino acids, OFR2 codes for a protein with a length of 290 amino acids. The protein derived from ORF1 includes an accumulation of hydrophobic amino acids as they are characteristic for membrane-embedded proteins. The detailed analysis of the distribution of the hydrophobic and hydrophilic amino acids by the programs PHD.HTM (Protein Science(1995)4:521-533) is shown in table 3. It is apparent therefrom that the protein contains six hydrophobic helix areas which extend through the membrane. Consequently, this protein is the searched for exporter of the amino acid L-lysine. The corresponding gene will therefore be designated below as lysE. In table 2, it is marked accordingly. ORF2 is transcribed in a direction opposite to ORF1. The sequence analysis shows that ORF2 has a high identity with regulator genes which are combined as a single family (Ann Rev Microbiol(1993) 597-626). Genes of this family regulate the expression processes of the various genes involved in catabolic or anabolic processes in a positive way. For this reason, ORF2 will below be designated as lysG (Govern=regulating). Because of the coordination and because lysE could be cloned (see a)) and subcloned (see b)) together with lysG, lysG is regulator of lysE and consequently also participates in the lysine export. The gene lysG and the amino acid sequence derived therefrom are also shown in SEQ ID

No(B)1 and table 2 and, respectively, SEQ ID No.[(B)]3 [and table 1].

Page 15, Sequence Protocol A:

Sequence protocol A:

SEQ ID [(A)1] 1: Nucleotide sequence of the coding DNA strand and the amino acid sequence of the Lysine-exporter LysE derived therefrom.

SEQ ID [(A)2] 2: Amino acid sequence of the Lysine-exporter LysE.

Sequence Protocol B:

SEQ ID No.(B)1: Nucleotide sequence of the anti-sense strand and Amino acid sequences of the Lysine-exporter-regulator $LysG_1$ derived therefrom and a ORF3.

SEQ ID No.(B)2: Amino acid sequence of the open reading frame (partial) ORF3

SEQ ID No.[(B)3] 3: Amino acid sequence of the Lysine exporter-Regulator $LysG_1$

Page 16, Table 1 - cancelled.

Pages 17 - 19, Table 2 - cancelled.

Page 20, Table 3 - cancelled.

The claims have been amended as follows:

1. A process for the microbacterial production of amino acids, comprising the steps of: providing a microbial organism having a certain export carrier activity and a certain export gene-expression,

increasing, selectively, one of

the export carrier activity of said microbial organism specific for a particular amino acid with an amino acid sequence as given in SEQ ID No. [(A)2 and in table 3, including any allele variation thereof,] 2 in accordance with the export carrier activity endogenous to said microbial organism,

and the export gene expression of said microbial organism specific for a particular amino acid with a nucleotide sequence of nucleotide 1016 to 1726 according to SEQ ID No. [(A)1 and table 2, or a DNA sequence with essentially the same effects,] 1 in accordance with the export gene expression endogenous to said microbial organism by means of one of the steps selected from the group of:

v) mutating the export carrier gene, such that an export carrier with increased export activity is generated,

vi) increasing the number of gene copies of the export carrier gene,

vii) modifying regulatory signals assigned to the export gene, and

viii) amplifying regulatory signals assigned to the export gene,

such that amino acids are produced by said microbial organism with increased efficiency.

4. A process according to claim 1, wherein the export gene expression of the export carrier is increased by increasing the number of gene copies, whereby the export carrier gene is expressed from the additional gene copies.

7. A process according to claim 5 [or 6], wherein the export gene is installed in a gene construct, which includes regulatory gene sequences [assigned] operably linked to the export gene.

8. A process according to claim 7, wherein the regulatory gene sequence includes a nucleotide sequence coding for the amino acid sequence as given in SEQ ID No.[(B)3] 3 [and in table 1 and the allele variations thereof] form nucleotide 1421-2293.

Delete claim 9

10. A process according [one of the claims 5 to 9] to claim 5, wherein a microorganism producing the respective amino acid is transformed with the gene construct including the export gene.

12. A process according to claim 10 [or 11], wherein, for the transformation, a microorganism is utilized in which the enzymes which participate in the synthesis of the corresponding amino acids are deregulated.

13. A process according to [one of claims 10 to 12] claim 10, wherein, for the transformation, a microorganism is util-

ized which contains an increased amount of the metabolites of the central metabolism.

14. A process according to [one of claims 4 to 13] claim 4, wherein the export gene is isolated from a microorganism strain of the type Corynebacterium.

15. A process according to [one of claims 1 to 14] claim 3, wherein the export gene sequence is identified by comparison with the sequence of an already known export gene.

16. A process according to claim 15, wherein the amino acid sequence derived from the export gene sequence to be identified is compared with the amino acid sequence given in SEQ ID No. 2 [(A)2 and in table 3 or the allele variation thereof].

17. A process according to [one of claims 1 to 16] claim 1, wherein the export gene expression is increased by amplifying the transcription signals.

18. A process according to [one of the 1 to 17] claim 1, wherein as export gene, a gene with a nucleotide sequence coding for the amino acid sequence given in SEQ ID No. 2 [(A)2 and in table 3 and the allele variations thereof] is utilized.

19. A process according to claim 18, wherein as export gene, one of the genes with the nucleotide sequence of nucleotide 1016 to 1726 according to SEQ ID No. 1 [(A)1 and table 2 and a DNA sequence with essentially the same effects] is utilized.

20. A process according to [one of the preceding claims 1 to 19] claim 1 for the manufacture of L-lysine.

43. The use of an export gene for increasing the amino acid production of microorganisms by:

i) constructing a gene construct including an export carrier gene,

ii) inserting said construct into a suitable vector,

iii) transforming a suitable host cell with said vector,

iv) cultivating said transformed host cell in a culture medium,

v) recovering the amino acid from the culture, and

vi) determining the desired amino acid amount.

Claim 44 cancelled

Claim 45 cancelled.

46. The use according to claim [45] 43, wherein the gene construct additionally carries regulatory gene sequences.

47. The use according to [one of the claims 43 to 46] claim 43, wherein an export gene from Corynebacterium is utilized.

48. The use according to [one of claims 43 to 47] claim 43, wherein Corynebacterium is used as amino acid producing microorganism.